

CLAIMS

1. A drug discovery process for identification of a small organic compound that is able to bind to a biological target molecule, the process comprising mutating a biological target molecule in such a way that at least one amino acid residue capable of binding a metal ion is introduced into the biological target molecule so as to obtain a metal ion binding site as an anchor point in the mutated biological target molecule.
2. A drug discovery process according to claim 1 further comprising
 - (a) contacting the mutated biological target molecule with a test compound which comprises a moiety including at least two heteroatoms for chelating a metal ion, under conditions permitting non-covalent binding of the test compound to the introduced metal ion binding site of the mutated biological target molecule, and
 - (b) detecting any change in the activity of the mutated biological target molecule or determining the binding affinity of the test compound to the mutated biological target molecule.
3. A drug discovery process according to claim 1 further comprising
 - (a) contacting the mutated biological target molecule with one or more members of a library of test compounds that comprise a moiety including at least two heteroatoms for chelating a metal ion, under conditions permitting non-covalent binding of at least a member of the library of test compounds to the introduced metal ion binding site of the mutated biological target molecule, and
 - (b) detecting any change in the activity of the mutated biological target molecule or determining the binding affinity of the test compound to the mutated biological target molecule.
4. A drug discovery process for identification of a small organic compound that is able to bind to a biological target molecule which has at least one metal ion binding site, the process comprising
 - (a) contacting the biological target molecule with a test compound which comprises a moiety including at least two heteroatoms for chelating a metal ion, under conditions permitting non-covalent binding of the test compound to the metal ion binding site of the biological target molecule, and
 - (b) detecting any change in the activity of the biological target molecule or determining the binding affinity of the test compound to the biological target molecule.
5. A drug discovery process for identification of a small organic compound that is able to bind to a biological target molecule which has at least one metal ion binding site, the process comprising

(a) contacting the biological target molecule with one or more members of a library of test compounds that comprise a moiety including at least two heteroatoms for chelating a metal ion, under conditions permitting non-covalent binding of at least a member of the library of test compounds to the metal ion binding site of the biological target molecule, and

(b) detecting any change in the activity of the biological target molecule or determining the binding affinity of the test compound to the biological target molecule.

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6. A drug discovery process according to any of claims 1-5 further comprising

(c) identifying the test compound that non-covalently binds to the biological target molecule.

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7. A drug discovery process according to any of claims 1-6 further comprising

(d) selecting two or more test compounds capable of forming a non-covalent binding to a biological target molecule, and capable of changing the activity of the biological target molecule or the binding affinity of the test compound to the biological target molecule

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to form a library of test compounds.

25 8. A drug discovery process according to any of claims 1-3 or 6-7 further comprising

(e) contacting the biological target molecule in wild-type, non-mutated form with at least one test compound determined to non-covalently bind the mutated biological target molecule in step (a), and

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(f) detecting any change in the activity of the biological target molecule or determining the binding affinity of the test compound to the biological target molecule.

35 9. A drug discovery process according to any of claims 1-3 or 6-7 further comprising

(e) contacting the biological target molecule in wild-type, non-mutated form with two or more members of a library of test compounds, wherein the test compounds in chelated form have been determined to non-covalently bind the mutated biological target molecule in step (a), and

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(f) detecting any change in the activity of the biological target molecule or determining the binding affinity of the test compound to the biological target molecule.

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10. A drug discovery process according to claims 8 or 9 further comprising

(g) identifying the test compound that interacts with the wild-type biological target molecule.

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11. A drug discovery process according to any of claims 1-7 further comprising

(e) contacting the biological target molecule in wild-type, non-mutated form with at least one test compound determined to non-covalently bind the mutated or non-mutated biological target molecule in step (a) but lacking a metal ion chelated thereto, and

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(f) detecting any change in the activity of the biological target molecule or determining the binding affinity of the non-chelated test compound to the biological target molecule.

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12. A drug discovery process according to any of claims 1-7 further comprising

(e) contacting the biological target molecule in wild-type, non-mutated form with two or more members of a library of non-chelated test compounds, wherein the test compounds in chelated form have been determined to non-covalently bind the mutated or non-mutated biological target molecule in step (a), and

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(f) detecting any change in the activity of the biological target molecule or determining the binding affinity of the non-chelated test compound to the biological target molecule.

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13. A drug discovery process according to claims 11 or 12 further comprising

(g) identifying the non-chelated test compound that interacts with the wild-type biological target molecule.

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14. A drug discovery process according to any of claims 8-13 further comprising

(a) identification of any binding or interaction between the non-chelated test compound and the wild-type biological target molecule.

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15. A drug discovery process according to any of claims 1-14, wherein the biological target molecule is a protein.

16. A drug discovery process according to claims 15, wherein the protein comprises an amino acid residue and wherein the metal ion binding site in the protein is introduced by amino acid substitution at or in the vicinity of

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1) a site where the binding of the test compound will interfere with the binding to another protein, for example a regulatory protein, or to a domain of the same protein;

- 2) a site where the binding of the test compound will interfere with the cellular targeting of the protein;
 - 3) a site where the binding of the test compound will directly or indirectly interfere with the binding of substrate or the binding of an allosteric modulatory factor for the protein;
 - 4) a site where the binding of the test compound may interfere with the intramolecular interaction of domains within the protein, for example the interaction of a regulatory domain with a catalytic domain;
 - 5) a site where binding of the test compound will interfere with the folding of the protein, for example the folding of the protein into its active conformation; or
 - 6) a site where the binding of the test compound will control the activity of the protein, for example by an allosteric mechanism.
17. A drug discovery process according to any of the preceding claims, wherein the metal ion binding amino acid residue in the biological target molecule is introduced by site-directed mutagenesis.
18. A drug discovery process according to any of the preceding claims, wherein the mutated biological target molecule is obtained as a recombinant expression product in purified or non-purified form.
19. A drug discovery process according to any of the preceding claims, wherein the mutated biological target molecule is obtained as a synthetic or semi-synthetic product.
20. A drug discovery process according to claim 15, wherein step (a) in any of claims 2-5 comprises the further step of determining, based on the three-dimensional structure of the specific protein in question or the primary structure of the specific protein together with a three-dimensional model of the class of proteins to which the specific protein belongs, the location of the metal ion binding amino acid residue in the mutated or non-mutated protein, and determining the location of at least one other amino acid residue in the vicinity of the metal ion binding amino acid residue.
21. A drug discovery process according to claim 15, wherein the binding of the test compound to the mutated or non-mutated protein in step (a) in any of claims 2-5 is determined using detection of any changes in the biological activity of the protein, competition with binding of a labelled ligand of the protein, or using a metal ion chelator which is in itself detectable or labelled with a detectable labelling agent.
22. A drug discovery process according to claim 19, wherein the amino acid residue in the vicinity of the metal ion binding amino acid residue is one which is capable of directly or indirectly binding at least one functional group of the test compound other than the metal ion.

23. A drug discovery process according to claim 22, wherein the amino acid residue capable of binding at least one functional group of the test compound other than the metal ion is detected using site-directed mutagenesis of at least one amino acid residue of the protein potentially involved in interaction with said functional group of the test compound other than the metal ion, followed by expression of the mutated protein in a suitable cell, contacting said cell or a portion thereof including the mutated protein with the test compound, and detecting any changes in the activity of the protein, determining any effect on binding in a competitive binding assay using a labelled ligand of the protein, or using a chelating agent which is in itself detectable or labelled with a detectable labelling agent.
24. A drug discovery process according to claim 22, wherein the amino acid residue capable of binding at least one functional group of the test compound other than the metal ion is detected by structural analysis employing i) a process involving crystallisation followed by X-ray, or ii) a process involving NMR.
25. A drug discovery process according to claim 15, wherein step (a) of any claims 2-5 comprises the further steps of improving the binding affinity of a metal ion chelate to the mutated or non-mutated protein, the method comprising
- (i) selecting a metal ion chelate with an activity to or a binding affinity to the mutated protein of 50 μ M or better as identified by the method of claim 21,
 - (ii) mapping the site of the protein to which the chelate binds using the method of claim 20, 23 and/or 24,
 - (iii) optionally locating at least one amino acid residue in the vicinity of the chelate,
 - (iv) altering one or more functional group of the chelate to optimise for direct or indirect interaction with said amino acid residue to generate a library of chelate derivatives,
 - (v) screening the derivatives of step (iv) by the method of claim 21,
 - (vi) selecting metal ion chelates having at least a two fold increase in activity or in binding affinity,
 - (vii) optionally repeating any one or a combination of two or more of steps (i)-(vi) one or more times to generate metal ion chelating compounds with an improved binding affinity for the mutated or non-mutated protein, and
 - (viii) optionally screening the thus selected metal ion chelates for binding to the wild-type protein by the method of claim 21,
 - (ix) optionally selecting metal ion chelates having at least an activity or a binding affinity to the wild-type protein of 50 μ M or better as identified by the method of claim 21, and
 - (x) optionally repeating any one or a combination of two or more of steps (viii)-(ix) one or more times to generate metal ion chelating compounds with an improved binding affinity for the wild-type protein.

26. A drug discovery process according to claim 15, wherein step (e) in any of claims 8-12 comprises the further steps of generating a library of test compounds which are derivatives of a test compound found to interact with the wild-type protein in step (e), each test compound in the library being provided with at least one functional group for direct or indirect interaction with at least one amino acid of the wild-type protein, which functional group differs from at least one functional group of each of the other test compounds, and screening the test compound library for compounds interacting with the wild-type protein.
27. A drug discovery process according to claim 15, wherein step (e) in any of claims 8-12 is performed by detecting any changes in the activity of the protein, detecting an effect on binding in a competitive binding assay using a labelled ligand of the protein, or using a chelating agent which is in itself detectable or labelled with a detectable labelling agent.
28. A drug discovery process according to claim 15, wherein step (e) in any of claims 8-12 comprises the further step of determining - based on the three-dimensional structure of the specific protein in question or the primary structure of the specific protein together with the three-dimensional model of the class of proteins to which the specific protein belongs, and based on the information provided by the method of claim 25 of the location of amino acid residues in the vicinity of the metal ion binding residue introduced in the mutated protein - the location of an amino acid residue in the wild-type protein binding at least one functional group of a test compound.
29. A drug discovery process according to claim 28, wherein the amino acid residue capable of binding at least one functional group of the test compound is detected using site-directed mutagenesis of at least one amino acid residue of the wild-type protein potentially involved in interaction with said functional group of the test compound, followed by expression of the mutated protein in a suitable cell, contacting said cell or a portion thereof including the mutated protein with the test compound, and determining any effect on binding using detection of any changes in the biological activity of the protein, a competitive binding assay using a labelled ligand of the protein, or using a chelating agent which is in itself detectable or labelled with a detectable labelling agent.
30. A drug discovery process according to claim 28, wherein the amino acid residue capable of binding at least one functional group of the test compound other than the metal ion is detected by structural analysis employing i) a process involving crystallisation followed by X-ray, or ii) a process involving NMR.
31. A drug discovery process according to any of claims 1-14, wherein the biological target molecule is selected from the group consisting of proteins, polypeptides, oligopeptides, nucleic acids, carbohydrates, nucleoproteins, glycoproteins, glycolipids, lipoproteins and derivatives thereof.

32. A drug discovery process according to claim 31, wherein the biological target molecule is a protein selected from the group consisting of membrane receptors, signal transduction proteins, scaffolding proteins, nuclear receptors, steroid
5 receptors, intracellular receptors, transcription factors, enzymes, allosteric enzyme regulator proteins, growth factors, hormones, neuropeptides or immunoglobulins.
33. A drug discovery process according to claim 32, wherein the protein is a
10 membrane protein.
34. A drug discovery process according to claim 33, wherein the biological target molecule is a membrane protein and the metal ion binding site in the biological target molecule is introduced in a ligand binding crevice of the membrane protein.
- 15 35. A drug discovery process according to claim 33, wherein the membrane protein is an integral membrane protein.
36. A drug discovery process according to claim 35, wherein the membrane protein comprises 1-14 transmembrane domains such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,
20 11, 12, 13 or 14 domains.
37. A drug discovery process according to claim 36, wherein the membrane protein is a receptor such as a tyrosine kinase receptor, e.g. a growth factor receptor such as the growth hormone, insulin, epidermal growth factor, transforming growth
25 factor, erythropoietin, colony-stimulating factor, platelet-derived growth factor receptor or nerve growth factor receptor (TrkA or TrkB).
38. A drug discovery process according to claim 36, wherein the membrane protein is a purinergic ion channel.
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39. A drug discovery process according to claim 36, wherein the membrane protein is a ligand-gated ion channel, such as a nicotinic acetylcholine receptor, GABA receptor, or glutamate receptor (NMDA or AMPA).
- 35 40. A drug discovery process according to claim 36, wherein the membrane protein is a voltage-gated ion channel, such as a potassium, sodium, chloride or calcium channel.
41. A drug discovery process according to claim 36, wherein the membrane
40 protein is a 7TM receptor, a G-protein coupled receptor, such as the acetylcholine receptors, ACTH receptors, adenosine receptors, adrenoceptors, anaphylatoxin chemotactic receptor, angiotensin receptors, bombesin (neuromedin) receptors, bradykinin receptors, calcitonin and calcitonin gene related peptide receptors, conopressin receptors, corticotropin releasing factor receptors, amylin receptors,
45 adrenomedullin receptors, calcium sensors, cannabinoid receptors, CC-chemokine

- receptors, cholecystokinin receptors, dopamine receptors, eicosanoid receptors, endothelin receptors, fMLP receptors, GABA_B receptors, galanin receptors, gastrin receptors, gastric inhibitory peptide receptors, glucagons receptors, glucagon-like I and II receptors, glutamate metabotropic receptors, glycoprotein hormone (e.g. FSH, LSH, TSH, LH) receptors, gonadotropin releasing hormone receptors, growth hormone releasing hormone receptors, growth hormone releasing peptide (Ghrelin) receptors, histamine receptors, 5-hydroxytryptamine receptors, leukotriene receptors, lysophospholipid receptors, melanocortin receptors, melanin concentrating hormone receptors, melatonin receptors, melanocortin receptors, neuropeptide Y receptors, neurotensin receptors, odor component receptors, opioid and opioid-like receptors, retinal receptors (opsins), orexin receptors, oxytocin receptors, parathyroid hormone and parathyroid hormone-related peptide receptors, P2Y receptors, pheromone receptors, platelet-activating factor receptors, prostanoid receptors, protease-activated receptors, secretin receptors, somatostatin receptors, tachykinin receptors, thyrotropin-releasing hormone receptors, pituitary adenylate activating peptide receptors, vasopressin receptors, vasoactive intestinal peptide receptors and virally encoded 7TM receptors; in particular galanin receptors, P2Y receptors, chemokine receptors, metabotropic glutamate receptors, melanocortin receptors, bombesin receptors, cannabinoid receptors, lysophospholipid receptors, fMLP receptors, neuropeptide Y receptors, tachykinin receptors, dopamine receptors, histamine receptors, 5-hydroxytryptamine receptors, histamine receptors, mas-proto-oncogene, acetylcholine, oxytocin, herpes virus encoded 7TM receptors, epstein-barr virus induced 7TM receptors, cytomegalovirus encoded receptors and bradykinin receptors; preferably galanin receptor type 1, leukotriene B4 receptor, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CX3CR1, mGLU-R1, mGLU-R2, m-GLU-R3, m-GLU-R4, m-GLU-R5, m-GLU-R6, m-GLU-R8, melanin concentration hormone receptors, melanocortin-1 receptor, melanocortin-3 receptor, melanocortin-4 receptor, melanocortin-5 receptor, bombesin receptor subtype 3, cannabinoid receptor 1, cannabinoid receptor 2, EDG-2, EDG-4, FMLP-related receptor I, FMLP-related receptor II, NPY Y6 receptor, NPY Y5 receptor, NPY Y4 receptor, NK-1 receptor, NK-3 receptor, D2 receptor (short), D2 receptor (long), Duffy antigen, U27, U28, UL33 and U78 from human cytomegalovirus, U12 and U51 from human herpes virus 6A, 6B or 7, ORF-74 from human herpes virus 8, Epstein Barr virus induced receptor -2, histamine H1 receptor, MAS proto-oncogene, muscarinic M1 receptor, muscarinic M2 receptor, muscarinic M3 receptor, muscarinic M5 receptor, oxytocin receptor, XCR1 receptor, RDC1 receptor, GPR12 receptor or GPR3 receptor.
- 40 42. A drug discovery process according to claim 36, wherein the membrane protein is a transporter protein, such as a GABA, monoamine, glutaminic acid or nucleoside transporter.
43. A drug discovery process according to claim 36, wherein the membrane
- 45 protein is a multidrug resistance protein, e.g. a P-glycoprotein, multidrug resistance

associated protein, drug resistance associated protein, lung resistance related protein, breast cancer resistance protein, adenosine triphosphate-binding cassette protein, Bmr, QacA or EmrAB/TolC pump.

- 5 44. A drug discovery process according to claim 36, wherein the membrane protein is a cell adhesion molecule, e.g. NCAM, VCAM or ICAM.
45. A drug discovery process according to claim 36, wherein the membrane protein is an enzyme such as adenylyl cyclase.
- 10 46. A drug discovery process according to claim 35, wherein the membrane protein is an orphan receptor.
47. A method of identifying a metal ion binding site in a biological target molecule, the method comprising
 - 15 (a) contacting the biological target molecule with a test compound which comprises a moiety including at least two heteroatoms for chelating a metal ion, under conditions permitting non-covalent binding of the test compound to the biological target molecule, and
 - 20 (b) detecting any change in the activity of the biological target molecule or determining the binding affinity of the test compound to the biological target molecule.
48. A method of identifying a metal ion binding site in a protein, the method comprising
 - 25 (a) analysing the nucleotide sequence of the gene coding for the protein in order to deduce the amino acid sequence,
 - (b) building a molecular model of the protein or a part of the protein based on the deduced amino acid sequence and the generic three-dimensional model of the class of proteins to which the specific protein belongs,
 - 30 (c) identifying the presence of amino acid residues such as, e.g., histidine, cysteine and/or aspartate residues, capable of binding a metal ion and located in suitable relative positions.
- 35 49. A method according to claim 47 or 48, wherein the test compound is contacted with two or more biological target molecules for identification of possible metal ion binding sites thereof.
50. A method of identifying a metal ion binding site in a protein, the method comprising
 - 40 (a) selecting a nucleotide sequence suspected of coding for a protein and deducing the amino acid sequence thereof,
 - (b) expressing said nucleotide sequence in a suitable host cell,
 - (c) contacting said cell or a portion thereof including the expressed protein
 - 45 with a test compound which comprises a moiety including at least two heteroatoms

for chelating a metal ion, under conditions permitting non-covalent binding of the test compound to the protein, and detecting any change in the activity of the protein or determining the binding affinity of the test compound to the protein, and

- (d) determining, based on the generic three-dimensional model of the class of proteins to which the protein or suspected protein belongs, at least one metal ion binding amino acid residue located in said protein to locate the metal ion binding site of said protein.

51. A method of mapping a metal ion binding site of a protein, the method comprising

- (a) contacting the protein with a test compound which comprises a moiety including at least two heteroatoms for chelating a metal ion, under conditions permitting non-covalent binding of the test compound to the protein, and detecting any change in the activity of the protein or determining the binding affinity of the test compound to the protein, and
- (b) determining, based on the primary structure of the specific protein in question and the generic three-dimensional model of the class of proteins to which the specific protein of step (a) belongs, at least one metal ion binding amino acid residue located in the membrane protein to identify the metal ion binding site of said membrane protein.

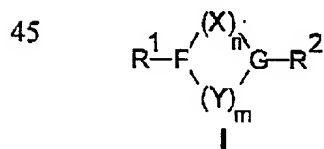
52. A drug discovery process according to any of claims 1-46 further comprising a method of any of claims 47-51.

53. A drug discovery process according to any of the preceding claims, wherein the test compound has a log K value in a range of from about 3 to about 18 such as, e.g. from about 3 to about 15, from about 3 to about 12, from about 4 to about 10, from about 4 to about 8, from about 4.5 to about 7, from about 5 to about 6.5 such as from about 5.5 to about 6.5.

54. A drug discovery process according to any of the preceding claims, wherein the test compound forms a chelate with a metal ion selected from the group consisting of Co, Cu, Ni, Pt and Zn including the various oxidation steps such as, e.g., Co (II), Co (III), Cu (I), Cu (II), Ni (II), Ni (III), Pt (II), Pt (IV) and Zn (II).

55. A drug discovery process according to any of the preceding claims, wherein the test compound has at least two heteroatoms, similar or different, selected from the group consisting of nitrogen (N), oxygen (O), sulfur (S), selenium (Se) and phosphorous (P).

56. A drug discovery process according to any of the preceding claims, wherein the test compound has the general formula I

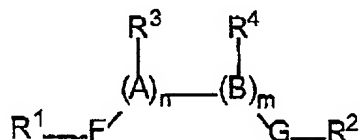


- 5 wherein F is N, O, S, Se or P; and G is N, O, S, Se or P;
 at least one of $(X)_n$ and $(Y)_m$ is present and if n is 0, then $-(X)_n-$ is absent, and if m
 is 0, then $-(Y)_m-$ is absent, and both n and m are not 0;
 R^1 and R^2 , which are the same or different, are radicals preferably selected from the
 group consisting of: hydrogen, a C_1 - C_{15} alkyl, C_2 - C_{15} alkenyl, C_2 - C_{15} alkynyl,
 10 aryl, cycloalkyl, alkoxy, ester, $-\text{OCOR}'$, $-\text{COOR}'$, heteroalkyl, heteroalkenyl,
 heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl or
 heteroaryl group, an amine, imine, nitro, cyano, hydroxyl, alkoxy, ketone,
 aldehyde, carboxylic acid, thiol, amide, sulfonate, sulfonic acid, sulfonamide,
 phosphonate, phosphonic acid group or a combination thereof, optionally
 15 substituted with one or more substituents selected from the same group as R^1 and/or
 a halogen such as F, Cl, Br or I;
 R' is hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl,
 substituted alkynyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl,
 heteroalkyl, substituted heteroalkyl, heteroalkenyl, substituted heteroalkenyl,
 20 heteroalkynyl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl,
 cycloalkenyl, substituted cycloalkenyl, heterocycloalkyl, substituted
 heterocycloalkyl, heterocycloalkenyl or substituted heterocycloalkenyl;
 R^1 and/or R^2 optionally forming a fused ring together with any of F, $(X)_n$ or a part
 of $(X)_n$, G, $(Y)_m$ or a part of $(Y)_m$ or R^1 and R^2 themselves forming a fused ring;
 25 X and Y are the same or different and have the same meaning as R' such as, e.g., -
 CH_2- , $-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-\text{S}-\text{CH}_2-$, $-\text{CH}_2-\text{N}-\text{CH}_2-$, $-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$,
 $-(\text{CH}_2)_d-$, $(Z)_e-(V)_f-(W)_g-(\text{CH}_2)_h-$, $-\text{CH}_2-\text{O}-\text{CH}_2-$, wherein
 each of Z and W are independently C, S, O, N, Se or P and
 V is $-\text{CH}-$ or $-\text{CH}_2-$;
 30 $(X)_n$ and/or $(Y)_m$ optionally being substituted with one or more substituents
 selected from the same group as R^1 and/or a halogen such as F, Cl, Br or I;

 n is 0 or an integer of 1-5,
 m is 0 or an integer of 1-5,
 35 e and/or g are an integer of 1-3,
 d, f and/or h are an integer of 1-7.

57. A drug discovery process according to claim 56, wherein the test compound
 has the general formula II

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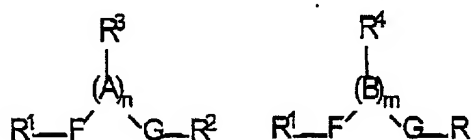
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- wherein F, G, R^1 and R^2 have the same meaning as in claim 56, R^3 and R^4 have the same meaning as R^1 and/or R^2 , and A and B have independently the same meaning as X and Y in formula I. n and m have the same meaning as in formula I except that n and m may be 0 at the same time and then the basic structure is



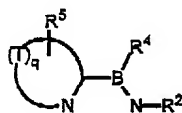
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and when n or m are 0, respectively, then the basic structures of formula II are

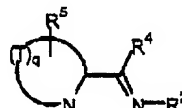


- 15 58. A drug discovery process according to claim 57, wherein F and/or G is nitrogen (N) and/or oxygen (O) and the test compound has the general formula III, IV, V, VI or VII:

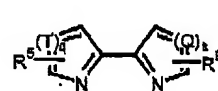
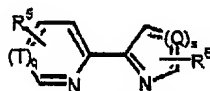
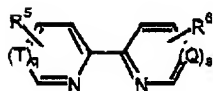
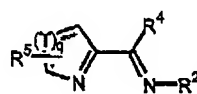
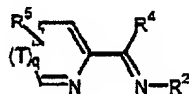
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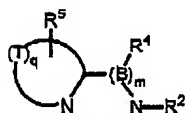
III A



III B

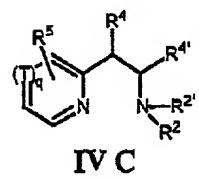
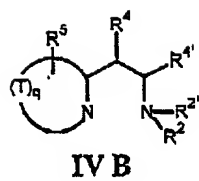


III C-G

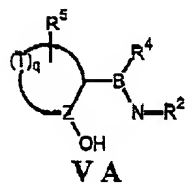


IV A

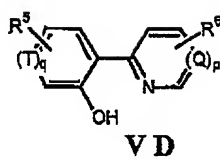
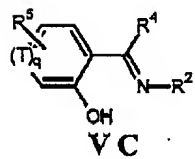
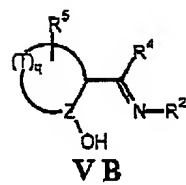
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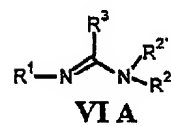
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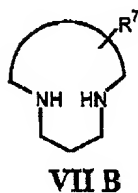
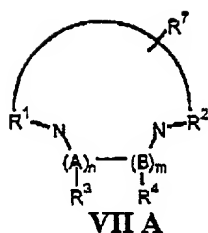


10



15





5

wherein T and Q are heteroatoms, and q and s independently are 0 or an integer of from 1 to 4;

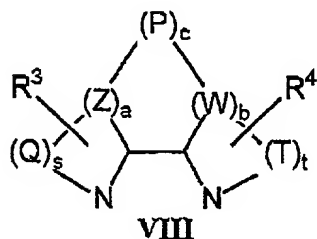
the meanings of q and s for q and/or s being 0 are the same as in Formula II for n and m;

10 a circle indicates a fused alkyl, alkenyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl or heteroaryl ring having from 3-7 atoms in the ring;

R^5 has the same meaning as R^1 and/or R^2 ;

15 and in Formulas III C-G, IV C and V C-D, T and/or Q may be placed anywhere in the cyclic system.

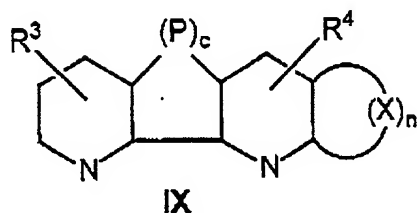
59. A drug discovery process according to claim 58, wherein the test compound has the general formula VIII



20

25 wherein R^3 , R^4 , Z, W and P are as defined herein before, a and/or b are an integer of 1-7 and c is 0 or an integer of 1-7, and each of Q and T is independently -CH- or -CH₂-, s is an integer of 1-7, and t is an integer of 1-7, are believed to be particularly suitable; when c is 0 in the above Formula VIII then $-(P)_c$ is absent, i.e. there is no bond between $(Z)_a$ and $(W)_b$.

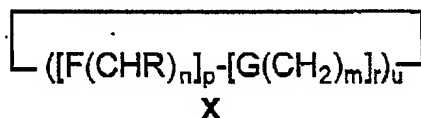
60. A drug discovery process according to claim 56, wherein the test compound has the general formula IX



5

wherein R^3 , R^4 , P, X and n are as indicated above, and r is 0 or an integer of 1-3, and when r is 0 then $-(P)_r$ is absent.

61. A drug discovery process according to claim 56, wherein the test compound has the general formula X



15 wherein F is N, O or S and G is N, O or S,
n is an integer from 1 to 5,

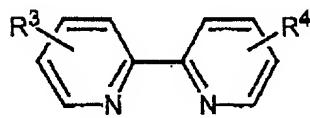
m is 0 or an integer from 1 to 5,

p and/or r are 0 or an integer from 1 to 8,

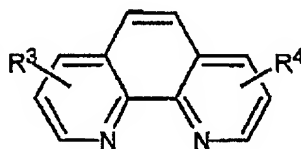
u is an integer from 1 to 8, and

20 R has the same meaning as R^1 in Formula I.

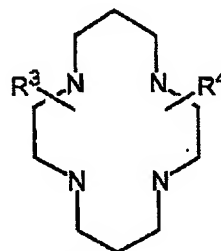
62. A drug discovery process according to claim 56, wherein the test compound has the general formula XI



XI a



XI b

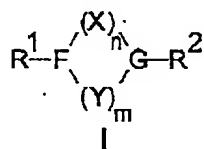


XI c

25

wherein R^3 and R^4 are as indicated above in formula I.

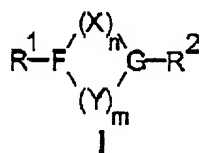
63. A drug discovery process according to any of claims 53-62, wherein the metal ion is one that binds to an amino acid residue containing a S, O, N, Se and/or P atom or with an aromatic amino acid residue.
- 5 64. A drug discovery process according to claim 63, wherein the amino acid residue is selected from the group consisting of Ser, Lys, Arg, Tyr, Thr, Trp, Phe, Asp, Glu, Asn, Gln, Cys and His, in particular Asp, Glu, Cys and His, preferably His.
- 10 65. A drug discovery process according to any of claims 53-64, wherein the metal ion is selected from the group consisting of aluminium, antimony, arsenic, astatine, barium, beryllium, bismuth, boron, cadmium, calcium, cerium, cesium, chromium, cobalt, copper, dysprosium, erbium, europium, gadolinium, gallium, germanium, gold, hafnium, holmium, indium, iridium, iron, lanthanum, lead, lutetium, magnesium, manganese, mercury, molybdenum, neodymium, nickel, niobium, osmium, palladium, platinum, polonium, praseodymium, promethium, rhenium, rhodium, rubidium, ruthenium, samarium, scandium, selenium, silicon, silver, strontium, tantalum, technetium, tellurium, terbium, thallium, thorium, thulium, tin, titanium, tungsten, vanadium, ytterbium, yttrium, zinc, zirconium, and oxidation states and isotopes thereof; in particular aluminium, antimony, barium, bismuth, calcium, chromium, cobalt, copper, europium, gadolinium, gallium, germanium, gold, indium, iron, lutetium, manganese, magnesium, nickel, osmium, palladium, platinum, rhenium, rhodium, rubidium, ruthenium, samarium, silver, strontium, technetium, terbium, thallium, thorium, tin, yttrium, zinc, and oxidation states or isotopes thereof; in particular cobalt, copper, nickel, platinum, ruthenium, and zinc, and oxidation states and isotopes thereof, preferably calcium (II), cobalt (II) and (III), copper (I) and (II), europium (III), iron (II) and (III), magnesium (II), manganese (II), nickel (II) and (III), palladium (II), platinum (II) and (V), ruthenium (II), (III), (IV), (VI) and (VIII), samarium (III), terbium (III), zinc (II), or isotopes thereof, preferably cobalt (II) and (III), copper (I) and (II), nickel (II) and (III), zinc (II) and platinum (II) and (V), or isotopes thereof.
- 25 66. A drug discovery process according to any of claims 53-65, wherein the test compound is a chelate like e.g. metal ion-phenanthroline complex, metal ion-bipyridyl complex and metal ion-1,4,8,11-tetraazacyclotetradecane complex such as, e.g., a Cu^{2+} -phenanthroline complex, a Zn^{2+} -phenanthroline complex, a Cu^{2+} -bipyridyl complex, a Zn^{2+} -bipyridyl complex, a Ca^{2+} -bipyridyl complex, a Cu^{2+} -1,4,8,11-tetraazacyclotetradecane, a Zn^{2+} -1,4,8,11-tetraazacyclotetradecane.
- 30 67. A chemical library comprising a plurality of test compounds of the following general formula I



- wherein F is N, O, S, Se or P; and G is N, O, S, Se or P;
 at least one of $(X)_n$ and $(Y)_m$ is present and if n is 0, then $-(X)_n$ is absent, and if m
 5 is 0, then $-(Y)_m$ is absent, and both n and m are not 0;
 R^1 and R^2 , which are the same or different, are radicals preferably selected from the
 group consisting of: hydrogen, a C_1 - C_{15} alkyl, C_2 - C_{15} alkenyl, C_2 - C_{15} alkynyl,
 aryl, cycloalkyl, alkoxy, ester, $-OCOR$, $-COOR$, heteroalkyl, heteroalkenyl,
 heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl or
 10 heteroaryl group, an amine, imine, nitro, cyano, hydroxyl, alkoxy, ketone,
 aldehyde, carboxylic acid, thiol, amide, sulfonate, sulfonic acid, sulfonamide,
 phosphonate, phosphonic acid group or a combination thereof, optionally
 substituted with one or more substituents selected from the same group as R^1 and/or
 a halogen such as F, Cl, Br or I;
 15 R' is hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl,
 substituted alkynyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl,
 heteroalkyl, substituted heteroalkyl, heteroalkenyl, substituted heteroalkenyl,
 heteroalkynyl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl,
 cycloalkenyl, substituted cycloalkenyl, heterocycloalkyl, substituted
 20 heterocycloalkyl, heterocycloalkenyl or substituted heterocycloalkenyl;
 R^1 and/or R^2 optionally forming a fused ring together with any of F, $(X)_n$ or a part
 of $(X)_n$, G, $(Y)_m$ or a part of $(Y)_m$ or R^1 and R^2 themselves forming a fused ring;
 X and Y are the same or different and have the same meaning as R' such as, e.g., $-$
 CH_2 -, $-CH_2-CH_2$ -, $-CH_2-S-CH_2$ -, $-CH_2-N-CH_2$ -, $-CH=CH-CH=CH$ -,
 25 $-(CH_2)_d$ -, $(Z)_e$ -, $(V)_f$ -, $(W)_g$ -, $(CH_2)_h$ -, $-CH_2-O-CH_2$ -, wherein
 each of Z and W are independently C, S, O, N, Se or P and
 V is $-CH$ - or $-CH_2$ -;
 $(X)_n$ and/or $(Y)_m$ optionally being substituted with one or more substituents
 selected from the same group as R^1 and/or a halogen such as F, Cl, Br or I;
 30
 n is 0 or an integer of 1-5,
 m is 0 or an integer of 1-5,
 e and/or g are an integer of 1-3,
 d, f and/or h are an integer of 1-7,
 35
 the test compounds being in the form of chelates formed between the test compound
 and a metal ion or atom selected from the group consisting of aluminium, antimony,
 arsenic, astatine, barium, beryllium, bismuth, boron, cadmium, calcium, cerium,
 cesium, chromium, cobalt, copper, dysprosium, erbium, europium, gadolinium,
 40 gallium, germanium, gold, hafnium, holmium, indium, iridium, iron, lanthanum,
 lead, lutetium, magnesium, manganese, mercury, molybdenum, neodymium, nickel,
 niobium, osmium, palladium, platinum, polonium, praseodymium, promethium,
 rhenium, rhodium, rubidium, ruthenium, samarium, scandium, selenium, silicon,
 silver, strontium, tantalum, technetium, tellurium, terbium, thallium, thorium,
 45 thulium, tin, titanium, tungsten, vanadium, ytterbium, yttrium, zinc, zirconium, and

- oxidation states and isotopes thereof; in particular aluminium, antimony, barium, bismuth, calcium, chromium, cobalt, copper, europium, gadolinium, gallium, germanium, gold, indium, iron, lutetium, manganese, magnesium, nickel, osmium, palladium, platinum, rhenium, rhodium, rubidium, ruthenium, samarium, silver, strontium, technetium, terbium, thallium, thorium, tin, yttrium, zinc, and oxidation states or isotopes thereof; in particular cobalt, copper, nickel, platinum, ruthenium, and zinc, and oxidation states and isotopes thereof, preferably calcium (II), cobalt (II) and (III), copper (I) and (II), europium (III), iron (II) and (III), magnesium (II), manganese (II), nickel (II) and (III), palladium (II), platinum (II) and (V), ruthenium (II), (III), (IV), (VI) and (VIII), samarium (III), terbium (III), zinc (II), or isotopes thereof, preferably cobalt (II) and (III), copper (I) and (II), nickel (II) and (III), zinc (II) and platinum (II) and (V), or isotopes thereof.

68. A chemical library comprising a plurality of test compounds of the following general formula I



- wherein F is N, O, S, Se or P; and G is N, O, S, Se or P;
at least one of $(\text{X})_n$ and $(\text{Y})_m$ is present and if n is 0, then $-(\text{X})_n-$ is absent, and if m is 0, then $-(\text{Y})_m-$ is absent, and both n and m are not 0;
 R^1 and R^2 , which are the same or different, are radicals preferably selected from the group consisting of: hydrogen, a C_1 - C_{15} alkyl, C_2 - C_{15} alkenyl, C_2 - C_{15} alkynyl, aryl, cycloalkyl, alkoxy, ester, $-\text{OCOR}'$, $-\text{COOR}'$, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl or heteroaryl group, an amine, imine, nitro, cyano, hydroxyl, alkoxy, ketone, aldehyde, carboxylic acid, thiol, amide, sulfonate, sulfonic acid, sulfonamide, phosphonate, phosphonic acid group or a combination thereof, optionally substituted with one or more substituents selected from the same group as R^1 and/or a halogen such as F, Cl, Br or I;
 R' is hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroalkyl, substituted heteroalkyl, heteroalkenyl, substituted heteroalkenyl, heteroalkynyl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heterocycloalkyl, substituted heterocycloalkyl, heterocycloalkenyl or substituted heterocycloalkenyl;
 R^1 and/or R^2 optionally forming a fused ring together with any of F, $(\text{X})_n$ or a part of $(\text{X})_n$, G, $(\text{Y})_m$ or a part of $(\text{Y})_m$ or R^1 and R^2 themselves forming a fused ring;
X and Y are the same or different and have the same meaning as R' such as, e.g., $-\text{CH}_2-$, $-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-\text{S}-\text{CH}_2-$, $-\text{CH}_2-\text{N}-\text{CH}_2-$, $-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$, $-(\text{CH}_2)_d-(\text{Z})_e-(\text{V})_f-(\text{W})_g-(\text{CH}_2)_h-$, $-\text{CH}_2-\text{O}-\text{CH}_2-$, wherein each of Z and W are independently C, S, O, N, Se or P and
V is $-\text{CH}-$ or $-\text{CH}_2-$;

(X)_n and/or (Y)_m optionally being substituted with one or more substituents selected from the same group as R¹ and/or a halogen such as F, Cl, Br or I;

n is 0 or an integer of 1-5,

5 m is 0 or an integer of 1-5,

e and/or g are an integer of 1-3,

d, f and/or h are an integer of 1-7,

the test compounds being in non-chelated form.

10

69. A chemical library comprising a plurality of metal ions selected from the group consisting of aluminium, antimony, arsenic, astatine, barium, beryllium, bismuth, boron, cadmium, calcium, cerium, cesium, chromium, cobalt, copper, dysprosium, erbium, europium, gadolinium, gallium, germanium, gold, hafnium, holmium, indium, iridium, iron, lanthanum, lead, lutetium, magnesium, manganese, mercury, molybdenum, neodymium, nickel, niobium, osmium, palladium, platinum, polonium, praseodymium, promethium, rhenium, rhodium, rubidium, ruthenium, samarium, scandium, selenium, silicon, silver, strontium, tantalum, technetium, tellurium, terbium, thallium, thorium, thulium, tin, titanium, tungsten, vanadium, ytterbium, yttrium, zinc, zirconium, and oxidation states and isotopes thereof; in particular aluminium, antimony, barium, bismuth, calcium, chromium, cobalt, copper, europium, gadolinium, gallium, germanium, gold, indium, iron, lutetium, manganese, magnesium, nickel, osmium, palladium, platinum, rhenium, rhodium, rubidium, ruthenium, samarium, silver, strontium, technetium, terbium, thallium, thorium, tin, yttrium, zinc, and oxidation states or isotopes thereof; in particular cobalt, copper, nickel, platinum, ruthenium, and zinc, and oxidation states and isotopes thereof, preferably calcium (II), cobalt (II) and (III), copper (I) and (II), europium (III), iron (II) and (III), magnesium (II), manganese (II), nickel (II) and (III), palladium (II), platinum (II) and (V), ruthenium (II), (III), (IV), (VI) and (VIII), samarium (III), terbium (III), zinc (II), or isotopes thereof, preferably cobalt (II) and (III), copper (I) and (II), nickel (II) and (III), zinc (II) and platinum (II) and (V), or isotopes thereof.

70. A chemical library according to claim 67 or 68, wherein the molecular weight of the individual test compounds is at the most 2000, log P is at the most 7, the number of hydrogen bond donors is at the most 10 and the number of hydrogen bond acceptors is at the most 15.

71. A chemical library according to claim 70, wherein the molecular weight of the individual test compounds is at the most 1500 such as, e.g., at the most 1000 or at the most 500; log P is at the most 6 such as, e.g., at the most 5; the number of hydrogen bond donors is at the most 8 such as, e.g., at the most 7, 6 or 5; and the number of hydrogen bond acceptors is at the most 13 such as, e.g., 12, 11 or 10

72. A chemical library according to any of claims 67-71 for use in a drug

discovery process according to any of claims 1-52.

73. Use of a test compound according to any of claims 53-66 in chelated form as either a stabilizing or as a destabilizing agent for di- or oligomerisation of a
5 biological target molecule.

74. Use according to claim 73, wherein the biological target molecule is a membrane protein.

- 10 75. Use according to claim 74, wherein the membrane protein is 7TM.

76. Use of a test compound according to any of claims 53-66 in pharmacological knock-out experiments employing a biological target molecule in which a silent metal ion binding site has been created without affecting the binding action of an
15 endogenous ligand for the biological target molecule with an aim of determining the effect of either an agonist or an antagonist on the physiological function of the metal ion site engineered receptor introduced into an animal by homologous gene replacement.

- 20 77. A method for characterising an orphan receptor, the method comprising

(a) mutating the orphan receptor in such a way that at least one amino acid residue capable of binding a metal ion is introduced into the orphan receptor so as to obtain a metal ion binding site as an anchor point in the mutated orphan receptor,

- 25 (b) contacting the mutated orphan receptor with a test compound which comprises a moiety including at least two heteroatoms for chelating a metal ion, under conditions permitting non-covalent binding of the test compound to the introduced metal ion binding site of the orphan receptor, and

- 30 (c) monitoring the binding of the test compound to the mutated orphan receptor by e.g. functional assays or through ligand binding assays.

78. A method according to claim 77 further comprising an optimization step in order to improve the affinity of the test compound.

- 35 79. Use of a test compound according to any of claims 53-66 as tracers in binding assays for orphan receptors.